I. OVERVIEW AND ACHIEVEMENTS

ENGINEERING BACTERIA TO PROMOTE ROOT GROWTH AND FIGHT SOIL EROSION

We have successfully created a bacterial system, Auxin, to combat soil erosion in three modules: Phyto-Route, Auxin Xpress, and Gene Guard. In doing so, we submitted several new funding parts to the Registry, including the first photoconvertible fluorescent reporter, dendr2a.

AIM - Rewire the E. coli chemotaxis pathway so our bacteria move to roots, where they can be naturally taken up by the plant. To do this, we used a root exudate responsive chemoreceptor from P. aeruginosa called PA2652.

MODELLING - The bacterial population dynamics is simulated for the capillary assay of chemotaxis (Fig. 4).

TESTING - A capillary assay was used to quantify the chemotactic response of E. coli with PA2652 towards malate. Fig. 5 shows the number of cells chemotaxing towards malate and Fig. 6 is a confocal micrograph of E. coli in Arabidopsis roots expressing gfp.

I. AUXIN XPRESS

Stimulating lateral root growth with E. coli

AIM - Engineer E. coli to secrete indole-3-acetic acid (IAA) to stimulate lateral root growth.

MODELLING - We predicted root growth patterns (Fig. 5) and combined this with our wetland data to simulate the effects of IAA on root growth.

TESTING AND RESULTS - The Salkowski assay (Fig. 6) and liquid chromatography mass spectrometry (LCMS) (Fig. 8) confirmed the presence of IAA in bacteria transformed with Auxin Xpress construct.

Our cells caused an IAA responsive Arabidopsis plant to fluoresce 2.9 times as brightly as the control (Fig. 10).

A photoconvertible reporter Dendr2a was used to show that our chassis is still alive inside the roots after 5 days (Fig. 11).

IV. APPLICATION

Combating soil erosion around the world.

PIPELINE - We have successfully demonstrated a proof of concept of our project. To develop our project further, we have drafted a ten year implementation pipeline including testing for safety and ecological impact and large-scale production trials.

SEED COAT - After meeting experts from Syngenta, Innocell and a patenting company, we found that the most versatile way of implementing our project is in the form of a seed coat (Fig. 17). Coated seeds are commercially used in agriculture and make for an easy-to-use product. We hope to use our project in countries around the world. We want to provide the product non-profit in less economically developed countries and finance operations through sales in developed countries. This follows the approach already used for the distribution of some pharmaceuticals.

Modelling guides positioning of engineered bacteria and the number of the bacteria required for one seed coat for the optimal IAA concentration (Fig. 18).

V. GENE GUARD

“For your cells only” - tackling horizontal gene transfer

AIM - Prevent the propagation of DNA constructs outside of the designated chassis.

DESIGN - Gene Guard uses a toxin/anti-toxin system in which genome-encoded anti-holin prevents plasmid-encoded holin and endolysin from lyzing the engineered chassis. However, in the event that the plasmid is transferred, lysis is induced in the recipient cell (Fig. 14).

When the plasmid is transferred into a wild type coli bacterium, holin will be produced. Without the action of genome-encoded anti-holin, holin forms pores in the inner cell membrane. Endolysin can then move through the membrane and induce lysis.

MODELLING - Gene Guard depends upon a critical ratio of holin to anti-holin expression. Anti-holin must be produced to an equal or greater concentration than holin in order to prevent the chassis from lysis. Holin must also be produced at sufficiently high concentrations to lyse recipient bacteria quickly. The required ratio of expression levels and hence the promoter and RBS strengths were modelled (Fig. 15).

TESTING - To demonstrate the need for a containment device like Gene Guard, we experimentally observed how long E. coli survive in soil. They were alive in non-sterile soil for over seven weeks and retained their plasmid in the absence of antibiotics (Fig. 18).

Our experiments show that Gene Guard can be used to create a safety engineering module.